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TITLE: UTILIZATION OF SIMIAN IMMUNODEFICIENCY VIRUS (SIV)
INFECTION FOR SUBHUMAN PRIMATES TO EVALUATE EXPERIMENTAL
CHEMOTHERAPY AND VACCINES

SUBTITLE: Determination of the ID50 of Frozen Stocks of Two
Strains of Simian Immunodeficiency Virus in Macaca
Mulatta

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FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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CONTRACT NO. DAMD 17-88-C-8160
FINAL REPORT
TASK ORDER II

Title:

Determination of the ID50 of Frozen Stocks of Two Strains of Simian Immunodeficiency Virus in Macaca mulatta.

Objective:

The purpose of this research is to determine the infectivity of the two strains of Simian Immunodeficiency Virus (SIVmac 251 and SIVmac 239) in rhesus macaques. The data obtained from this study will provide information for the design of future lentiviral vaccine studies involving rhesus macaques and one of the above strains.

Study Design:

On April 17, 1991, 38 candidate rhesus monkeys between the age of 18 months and 4 years were selected for the study. Two of these animals served as replacement if needed. These animals were observed daily and health observations were recorded during the entire baseline period, including body weights, body temperature, heart beats as well as clinical evaluations (CBC and SMAC). All 38 animals were tested negative for SRV (type D retrovirus). The virus inoculum was received from the Department of the Army in June, 1991. Serum from each animal was collected and frozen for serologic evaluation as baseline control before the inoculation on June 25, 1991.

Experimental Design:

1. Animal Inoculation:

Animals were inoculated by Dr. Rick Miller, D.V.M. on June 25, 1991. All animals received 1ml of the respective inoculum via the saphenous vein as designated in the table below.

Viral Inoculum	Group	Log 10 Dil. of Inoculum	No. of Anim. Per Group	Animals Designation
SIVmac 251	1	10 ^{-2.5}	3	5544, 5530, 88C
	2	10 ^{-3.5}	4	5606, 92B, 5511, 5600
	3	10 ^{-4.5}	4	81C, 5316, 3D, 105C
	4	10 ^{-5.5}	4	5320, 5601, 110C, 5567
	5	10 ^{-6.5}	3	5568, 26C, 189B
SIVmac 239	1	10 ^{-3.5}	3	150B, 5561, 146B
	2	10 ^{-4.5}	4	5549, 131B, 5593, 5543
	3	10 ^{-5.5}	4	101C, 5575, 5550, 5309
	4	10 ^{-6.5}	4	5319, 5605, 20D, 209B
	5	10 ^{-7.5}	3	5603, 117B, 114C

In addition to the cocultivation at PRL, a schedule of shipment of whole blood specimens from monkeys to be sent to sponsor/collaborators via overnight delivery was as follows:

Date	Animal Group	PRL	Walter Reed Specimen
6/25/91 Day 0	All animals, groups 251 & 239	Plasma	
7/2/91 Week 1	All animals, groups 251 & 239	CBC	
7/9/91 Week 2	All animals groups 251 & 239	Cocult	
7/22/91 Week 4	251 animals only		10ml heparinized blood
7/29/91 Week 5	239 animals only		10ml heparinized blood
8/5/91 Week 6	251 animals only, groups 3, 4, 5		10ml heparinized blood
8/12/91 Week 7	239 animals only, groups 3, 4, 5		10ml heparinized blood
8/19/91 Week 8	251 animals only, groups 3, 4, 5		10ml heparinized blood
8/26/91 Week 9	239 animals only, groups 3, 4, 5		10ml heparinized blood

2. Virus Isolation:

Methods:

At day 14 post inoculation, virus isolation was performed. Peripheral blood mononuclear cells (PBMC) were separated from heparinized whole blood using lymphocyte separation medium (LSM) gradients. The PBMC were cocultivated with 2×10^6 /ml CEMX174 (B cell and T cell hybrid) in complete RPMI 1640 medium supplemented with 10% Fetal Bovine Serum, 20mM L-glutamine, penicillin (100 μ g/ml)/streptomycin (100 μ g/ml). Cell free culture fluids were harvested once weekly and assayed for reverse transcriptase (RT) activity. All cultures were maintained for eight weeks or until positive for RT activity.

Results:

The results are summarized in the table below:

Virus Inoculum	Log 10 dilution	Animal Number	Isolation
SIVmac 251	10 ^{-2.5}	5544	+
		5530	+
		88C	+
	10 ^{-3.5}	92B	+
		5606	+
		5511	+
		5600	+
	10 ^{-4.5}	81C	+
		5316	-
		3D	-
		105C	+
	10 ^{-5.5}	5320	-
		5601	+
		110C	-
	10 ^{-6.5}	5567	-
		5568	-
		26C	-
		189B	-
SIVmac 239	10 ^{-3.5}	150B	+
		5561	+
		146B	+
	10 ^{-4.5}	5549	+
		131B	+
		5593	+
		5543	+
	10 ^{-5.5}	101C	-
		5575	-
		5550	+
		5309	-
	10 ^{-6.5}	5319	-
		5605	-
		20D	+
	10 ^{-7.5}	209B	-
		5603	-
		117B	-
		114C	-

One week after cocultivation with CEMX174 cells, animals 131B and 5549 of the SIVmac 239 group ($10^{-4.5}$) were detected virus positive by RT assay. At week 3, animals 88C (SIVmac 251, $10^{-2.5}$), 5606 (251, $10^{-3.5}$) and 20D (239, $10^{-6.5}$) showed positive RT activity. By the fourth week, animals of the 251 group 5544, 5530 ($10^{-2.5}$), 5600 ($10^{-3.5}$), and 81C ($10^{-4.5}$) were detected RT positive. Animals 92B and 5511 ($10^{-3.5}$) of the 251 group and 150 B, 5561, 146B ($10^{-3.5}$) and 5550 ($10^{-5.5}$) of the 239 group turned positive at sixth and seventh week. During the final week of cocultivation, 105C ($10^{-4.5}$) and 5601 ($10^{-5.5}$) of the 251 group were virus positive. The rest of the animals remained virus negative by RT assay.

Animal 131B (239, $10^{-4.5}$) was necropsied on July 14, 1991 by Dr. Lackner, PRL pathologist. At the time of necropsy, 10ml heparinized blood was collected for virus isolation. The PBMC were separated and cocultivated with CEMX174 cells for SIV. Two week post inoculation and at the time of death, this animal was determined virus positive by reverse transcriptase assay. Also at the time of necropsy, swabs of jejunum, stomach, colon and pericardium were cultured for Shigella and other pathogenic microorganisms. Only Shigella flexneri serogroup B was isolated in the jejunum.

TASK ORDER II-EXTENSION

In October, 1991, the Department of the Army requested to extend the Task Order II including challenge of the animals that were determined to be SIV negative after the first inoculation in June, 1991.

Before the virus challenge, all the study animals were bled for a final bleed (10ml and 5ml heparinized blood). The 10ml blood specimens were shipped to Dr. Lewis, Henry Jackson Foundation. The 5ml specimens were shipped to Dr. Ansari of Emory University Medical Center in Atlanta, Georgia, as requested by Dr. Lewis.

After the final bleed, inguinal lymph node biopsies were performed on the 251 monkeys: 5316, 105C, 3D, 5320, 5601, 5567, 5568, 26C, 81C, 189B and 110C, and on the 239 monkeys: 101C, 5575, 5550, 5309, 5319, 5605, 20D, 209B, 5603, 117B and 114C. The biopsy tissues were shipped to Dr. Lewis.

The SIV positive animals 88C, 92B, 5605, 81C, 5511 and 5600 were necropsied. The sterile tissues collected from axillary lymph nodes, inguinal lymph nodes, mesenteric lymph nodes, spleen and thymus. The remaining tissues were fixed in formalin. All tissues were sent to Dr. Lewis.

The animals 5544, 5530, 105C and 5601 of the SIVmac 251 group were held for six months to observe for disease outcome. These animals were bled at monthly intervals and the heparinized blood specimens were shipped to Henry Jackson Foundation. Additional peripheral blood and sera were collected for CBC and clinical analyses each time. The remaining confirmed SIV positive monkeys 150B, 5561, 146B, 5549, 5593, 5550, 20D, 101C and 5543 were returned to PRL for disposition.

The SIV-negative animals from the 251 and 239 groups were challenged on January 27, 1992. The animals included:

SIVmac 251

5316
3D
5320
110C
5567
5568
26C
189B

SIVmac 239

5575
5309
5319
5605
209B
5603
117B
114C

The viral inoculum was diluted as follows:

SIVmac 251-4/19/91 dilutions:

Dilution	1:30	1:300	1:3000
PBS	2.9ml	9.0ml	9.0ml
Virus	*0.1ml	\$1.0ml	1.0ml

SIVmac 239-10/1/90 dilutions:

Dilution	1:30	1:300	1:3000	1:30,000
PBS	2.9ml	9.0ml	9.0ml	9.0ml
Virus	*0.1ml	§1.0ml	§1.0ml	1.0ml

*from virus obtained from R. Desrosiers

§from previous dilution

Each animal received 1ml of the viral inoculum via the left saphenous vein. The 251 animals received 1ml of the 1:3000 dilution each, while the 239 group animals received the 1:30,000 dilution each. Ten ml of heparinized blood was drawn from each animal at the time of challenge to be sent to Henry Jackson Foundation. Additional peripheral blood was collected for CBC and clinical chemistry. Following the virus challenge, all the animals were held for two months. Weekly blood draw of 10ml heparinized blood from each animal was sent to Henry Jackson Foundation.

The table below shows the schedule of the bleed:

Week	Date	HJF 10ml hep. blood	CBC	SMAC	Physical	O&P
0	*1/27/92	X	X	X	X	X
1	2/3/92	X				
2	2/10/92	X	X	X		
3	2/18/92	X	X		X	
4	2/24/92	X	X	X	X	X
5	3/2/92	X	X		X	
6	3/9/92	X	X	X	X	
7	3/16/92	X	X			
8	3/23/92	X	X		X	

*day of challenge

Animals 5601 and 189B of the 251 group were necropsied before the end of the two month period due to declining health. Sterile tissues such as spleen, thymus, axillary, inguinal and mesenteric lymph nodes, heparinized blood were collected for

Dr. Lewis. The remaining tissues were fixed in formalin and shipped to Dr. Lewis. A final CBC, clinical chemistry and blood cultures were also performed.

Four months after the challenge, the animals were scheduled to have axillary lymph node biopsies. All the challenged animals except 5568 underwent the procedure. Physicals and blood chemistry were done before the biopsy. Each biopsy tissue was divided for sterile specimen, frozen and paraffin blocks. Animal 5568 was necropsied due to declining health.

The remaining SIVmac 251 animals 3D, 26C, 110C, 5316, 5320, 5567, 5544, 5530 and 105C were requested by the Department of the Army to be released and transported to Henry Jackson Foundation upon completion of the study contract.

The remaining SIVmac 239 animals 5603, 117B, 5575, 5309, 5319, 5605 209B and 114C were necropsied. Weights of pooled axillary lymph nodes, pooled inguinal lymph nodes, the ileocecal lymph node, and the spleen were recorded. Tissue samples of spleen, thymus, axillary lymph nodes, inguinal lymph nodes, mesenteric lymph nodes and the ileocecal valve were collected sterile. Small duplicate samples of the same tissues were processed for both frozen and paraffin blocks. A complete set of tissues including the CNS and GI tract was fixed in formalin and was shipped to Dr. Lewis with the rest of the sterile tissues.